(12) UK Patent Application (19) GB (11) 2 111 201 A

(21) Application No 8235151
(22) Date of filing 9 Dec 1982
(30) Priority data
(31) 8137366
(32) 10 Dec 1981
(31) 8205743
(32) 26 Feb 1982
(33) United Kingdom (GB)
(43) Application published
29 Jun 1983
(51) INT CL³
G01N 33/54
(52) Domestic classification
G1B BB
C6F HA2
U1S 1296 C6F G1B
(56) Documents cited
GB A 2086041
GB A 2079313
GB A 2034886
EP A1 0044219
(58) Field of search
G1B
C6F
(71) Applicant
Celltech Limited,
(Great Britain),
244—250 Bath Road,
Slough,
Berkshire,
SL1 4DY
(72) Inventors
William Michael Hunter,
Keith James
(74) Agent and/or address for service
Celltech Limited,
P. E. Crawley,
244—250 Bath Road,
Slough,
Berkshire,
SL1 4DY

(54) Immunoassay of human luteinising hormone

(57) An improved immunoassay method for the estimation of human luteinising hormone (LH) present in a sample comprises,

(a) binding LH present in the sample to both a labelled receptor and an unlabelled receptor to form a solid labelled complex, and

(b) analysing for either labelled receptor bound in the solid labelled complex or residual unbound labelled receptor,

wherein the labelled receptor is a labelled monoclonal antibody to LH. Such monoclonal antibodies to LH are included within the scope of the invention, and in a preferred embodiment a conformational monoclonal antibody is provided e.g., anti-human LH No. ESI.

GB 2 111 201 A

2111201

Fig.1.

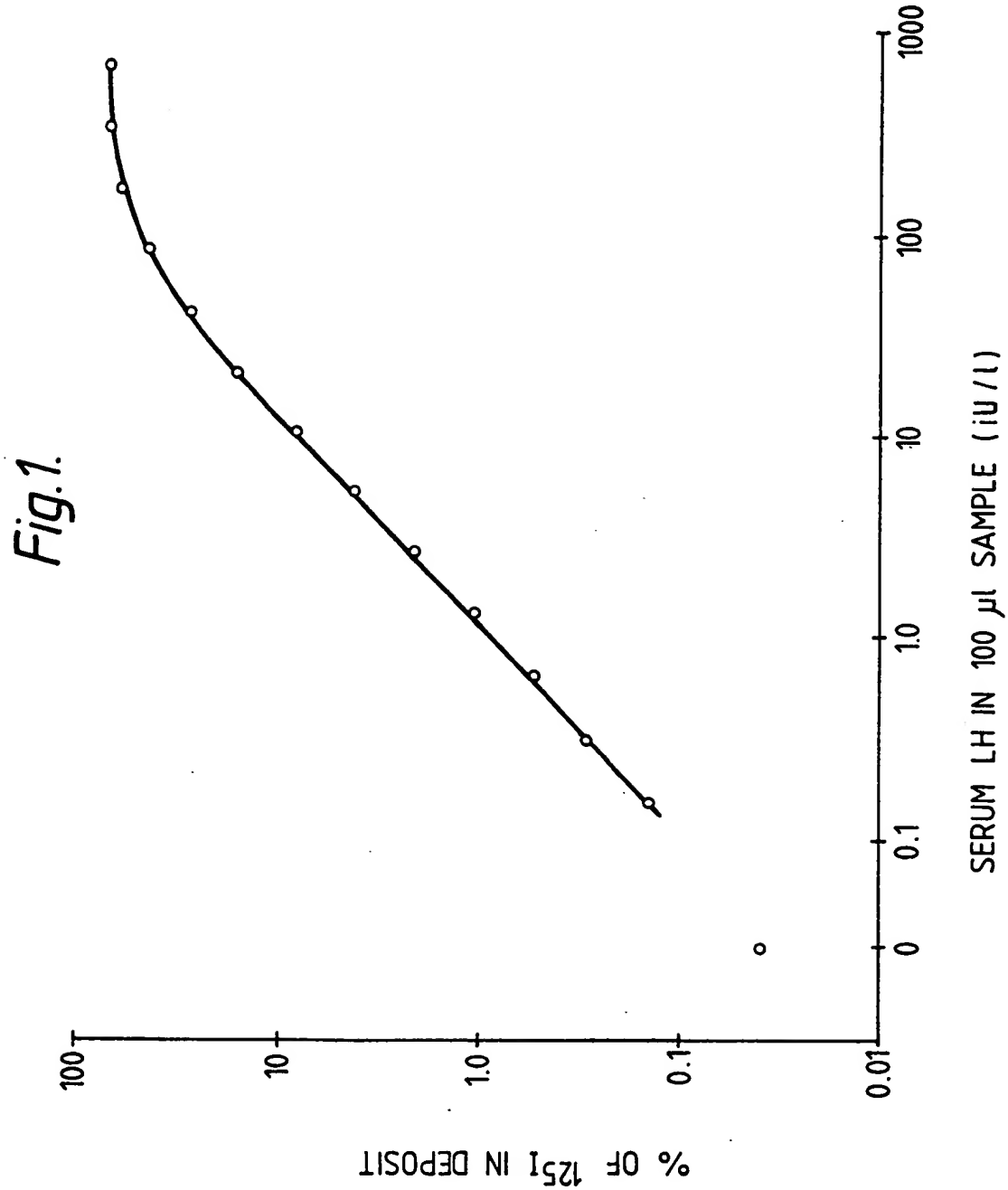
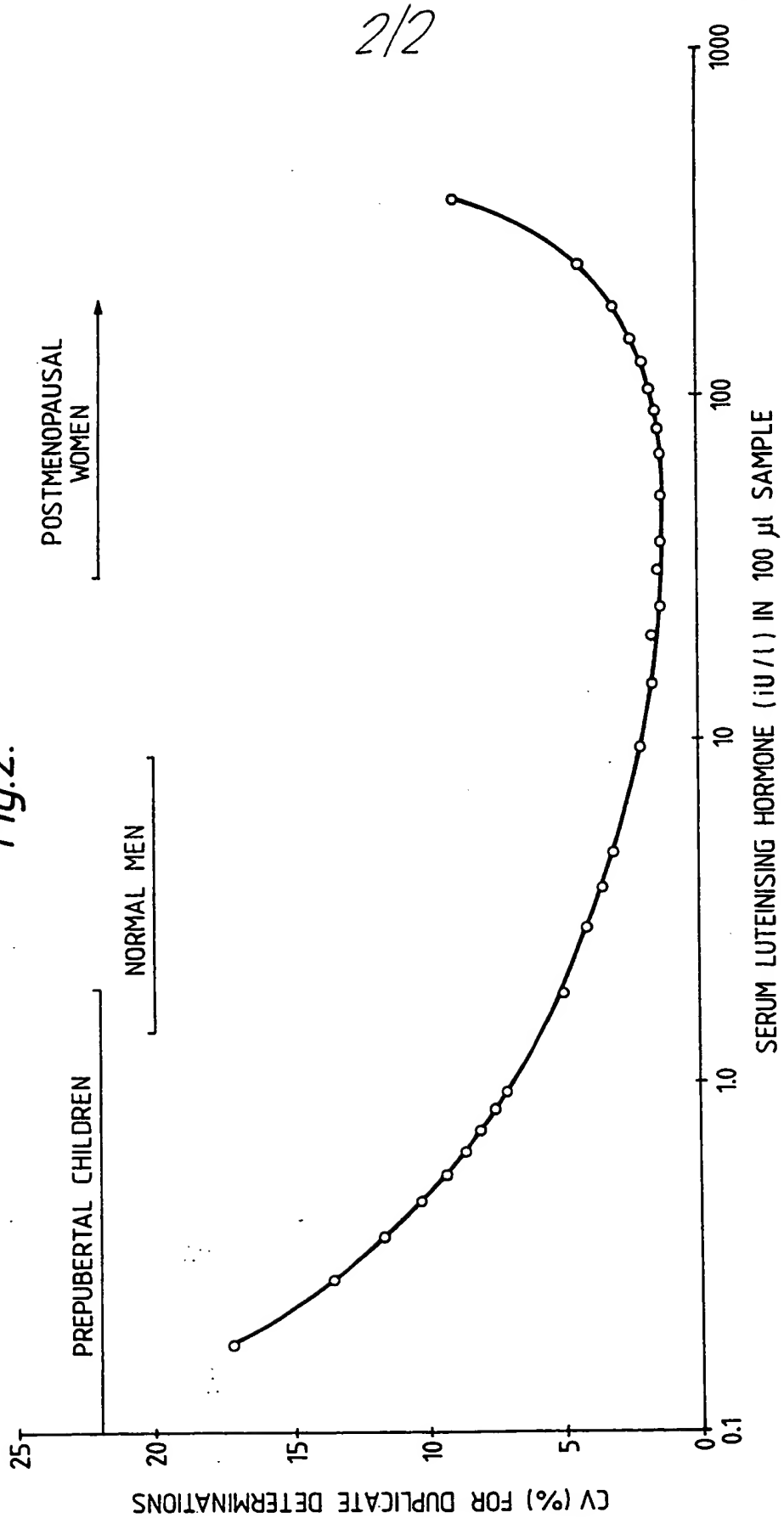


Fig.2.


CV (%) FOR DUPLICATE DETERMINATIONS
25
20
15
10
5
0
0.1
1.0
10
100
1000
SERUM LUTEINISING HORMONE (iU/l) IN 100 µl SAMPLE
PREPUBERTAL CHILDREN
NORMAL MEN
POSTMENOPAUSAL WOMEN

SPECIFICATION

Immunoassay of human luteinising hormon

The present invention relates to an immunoassay method for the estimation of human luteinising hormone (LH).

The measurement of LH levels in biological fluids has a number of clinical applications. For example, serum LH measurements may be used to assist in:

a) The differentiation of prepubertal from pubertal patients. The former have basal serum LH levels below 2 IU per litre whilst the latter generally have serum values above this level.

b) The differentiation of the pathophysiological states found amongst patients with amenorrhoea, expecially secondary amenorrhoea. Patients with basal LH levels above 30 IU per litre are likely to have ovarian failure associated with, for example, a postmenopausal state; those with basal levels in the range 2 to 20 IU per litre have at least partial ovarian and pituitary function, whilst those with basal serum LH levels below 2 IU per litre have hypofunction at the pituitary or, more usually, at the hypothalamic level.

c) Discovery whether the positive feedback mechanism which gives rise to a major surge of LH secretion in the middle of the menstrual cycle and which is characteristic of normal hypothalamic-pituitary-ovarian function in women of reproduction age is intact, and

d) Monitoring the timing of the events relatng to naturally occuring, or medically induced ovulation in women.

It is the aim of the present invention to provide an improved immunoassay method for the estimation of LH which has a wide working range, a favourable lower detection limit and a short turnover time.

According to the present invention an immunoassay method for the estimation of human luteinising hormone (LH) present in a sample comprises:

a) binding LH present in the sample to both a labelled receptor and an unbalanced receptor to form a solid labelled complex, and

b) analysing for either labelled receptor bound in the solid labelled complex or residual unbound labelled receptor,

wherein the labelled receptor is labelled monoclonal antibody to LH.

Accordingly also the invention comprises a monoclonal antibody produced by a hybrid cell line wherein the antibody has specificity for human luteinising hormone (LH).

The monoclonal antibodies of the present invention may be obtained from any appropriate hybrid cell line, and are preferably derived from a hybrid myeloma produced by fusion of a parent cell line e.g. NS1 (abbreviated from P3-NS1/1-Ag4—1 [G. Kohler, S. C. Howe and C. Milstein, European Journal of Immunology (1976) *6*, 292—295] available on request from the MRC Laboratory of Molecular Biology, Hills Road, Cambridge) with spleen or other immunocyte cells from mice or rats which have been immunised with LH. In a particularly preferred embodiment of the invention the MAb is an antibody which reacts with an epitope which includes structures on both the the alpha and beta subunits of intact LH, i.e. a conformational anti LH MAb. Expecially, the MAb is a MAb having the identifying characteristics of the MAb designated ES1; for example, the MAb ES1 present in the ascites fluid of a mouse which has been innoculated with a hybrid cell line, anti-human LH No ES1, available from Dr K. James, Department of Surgery, Edinburgh University, Wilkie Laboratories, Medical Building, Teviot Place, Edinburgh EH8 9AG, prepared by fusing the parent cell line NS1 with spleen cells from a mouse immunised with LH. This hybrid cell line is also available from the Collection Nationale de Cultures de Microorganisms, Pasteur Institute, Paris under deposition No CNCM I—185 (deposited on 24 February 1982).

Conformational MAbs such as ES1 are particularly desirable for use in the immunoassay method of the present invention. These antibodies selectively bind the intact hormone rather than either of the constituent alpha and beta subunits, and thus the assay will measure preferentially the intact hormone and will respond poorly to subunits of LH or other glycoprotein material such as TSH or FSH, present in the sample.

In this latter respect, it has been found that when the ES1 MAb is used in the method of the invention, the response curves given by the alpha and beta subunits, though parallel to the response curve for intact LH, indicate potencies of 2.5% and 4.5% respectively of that of the intact hormone. These determinations were carried out using standard preparations of intact human LH and of the alpha and beta subunits, and it has been further found that the low responses of the alpha and beta subunit preparations are attributable to contamination by intact LH.

Preferably also the MAb comprises an IgG antibody, having desirable stability characteristics when the MAb is used in labelled form in the immunoassay method of the invention. In this respect it has been found that the MAb ES1 is an antibody of class IgG1.

Any suitable label may be used on MAb of the invention including, for example, enzymes, fluorophores and chemiluminescent compounds. Preferably, however, the MAb is labelled with a radioactive tag, especially iodine 125.

Generally the unlabelled receptor will be a material which binds to LH to form a solid unlabelled complex. Preferably these materials comprise one or more of the following:

i) antiserum (especially IgG) to human chorionic gonadotrophin (hCG);

ii) antiserum to LH;

iii) a monoclonal antibody to hCG, or

iv) a monoclonal antibody to LH (provided that the MAb reacts with a different epitope than the MAb used as labelled receptor),

convalently or otherwise bound to a solid

phase support, for example, cellulose, but especially an agarose (Sepharose, Trade Mark), a cross-linked dextran (Sephadex, Trade Mark) or a copolymer of agarose and acrylamide (Sephacryl, Trade Mark), with Sephacryl being particularly preferred. Preferably the antibody/antisera is bound to Sephacryl using the periodate oxidation procedure hereinafter described. The advantages of using such a solid phase support are that the support can form substantially stable suspensions in aqueous media, can be conveniently handled during manipulations such as pipetting and centrifuging and exhibits low non-specific adsorption properties, or can be treated so that it exhibits such adsorption properties.

When the unlabelled receptor is antiserum or a monoclonal antibody to hCG this may be antiserum or a monoclonal antibody to either the alpha or beta subunits of hCG or to the whole molecule. Thus, for example, it may be desirable to use a general purpose antiserum or a monoclonal antibody to the alpha subunit which may have uses in immunoassays for other glycoprotein hormones besides LH. Alternatively, for example antiserum or a monoclonal antibody to the beta subunit or the whole molecule may provide more specific reagent for use in immunoassay of LH.

Step (a) of the immunoassay method of the present invention may be effected by any one of at least three alternative procedures. In the first the LH is bound consecutively to the labelled MAb and the unlabelled receptor in a two-stage process. In the second the LH is bound consecutively to the unlabelled receptor and the labelled MAb, again in a two-stage process. In the third the LH is bound to both the labelled MAb and the unlabelled receptor in one binding step, i.e. a single-stage process.

In all three of the above procedures the LH is generally contained in a sample of biological fluid, such as serum.

The LH is preferably bound to the MAb and the unlabelled receptor by incubation in an aqueous medium. Once the LH has been bound to both the labelled MAb and the unlabelled receptor, a solid labelled complex results.

After incubation in the aqueous medium, the solid complex comprising bound labelled receptor is then usually separated from the aqueous medium which contains in solution residual unbound labelled receptor, to facilitate analysis of the labelled receptor either in the bound complexed state or in the residual unbound form in solution in the aqueous medium. Separation of the solid complex from the aqueous medium is effected preferably by the sucrose layering technique describ d in UK Patent No 1566098 and US Patent No 4125375. Analysis of the labelled MAb may be achieved by determining th amount of residual label present in the aqueous medium or preferably the amount of label pres nt in the separated solid compl x.

It is a particular advantage of the sucrose layering separation system that the radioiodinated MAb prepared from the preferred hybrid cell line ES1 displays very low adsorption characteristics therein. For example, when the Sephacryl (TM) S300—coupled sheet anti hCG is used in the immunoassay of the present invention on a sample that is free of LH, only 0.03% of added ^{125}I—MAb ES1 is present in the bound fraction after two sucrose washes. Furthermore this adsorption is to the tube surface and virtually in such adsorption occurs with the solid-phase reagent.

By comparing the result obtained from the above analysis with a separately prepared standard curve of LH concentration against labelled MAb present in the solid complex the original concentration of the LH in the sample may be ascertained.

The method of the present invention offers significant advantages (wide working range, low detection limit, short turnover time) over many of the previous "two site" immunoassays for LH. Moreover, the use of a labelled MAb is particularly advantageous in the immunoradiometric assay (IRMA) of LH. In the IRMA technique the MAb is labelled with a radioactive tag, expecially iodine-125. This, together with the unlabelled solid phase receptor, is then attached to LH to form a radioactive solid complex. Separation of the radioactive solid complex from the reaction medium is followed by analysis of the solid complex or the medium, by a gamma counter, for the level of radioactivity. From this result, the concentration of the LH in the original sample may be ascertained.

The present inventors have found that by using the IRMA technique in the method of the present invention, a single general purpose assay for LH is provided which can be used for all present clinical purposes and which yields the required results within one working day.

The major use of the present method will be in clinical laboratories. It is usual for such laboratories to obtain the materials to be used in a given assay procedure in the form of an assay kit.

Accordingly, the present invention also provides an assay kit for use in the estimation of LH comprising:

a) a labelled monoclonal antibody to LH, and

b) an unlabelled receptor which binds to LH to form a solid unlabelled complex.

Preferably the labelled MAb and the unlabelled receptor will be those listed above as preferred for use in the method of the present invention.

Optionally the assay kit may also contain LH standards, particularly samples of the International Standard for LH (preparation 68/40, available 10 from th National Institute of Biological Standards and Controls, Holly Hill, London NW3 6RB) or a working standard calibrat d against this. In addition the kit may also include various other reagents and/or equipment employed in the preferred embodiments of the present process. For example, there may be

included a sucrose solution for use in the preferred 15 separation step (the sucrose layering system of UK 1,566,098 and US 4,125,375), buffer solutions, such as Tris phosphate 0.25M pH 8.5 containing 2% (w/v) serum e.g. bovine or sheep serum, and 0.1% (w/v) sodium azide, for use as aqueous media in the incubation of LH with its receptors syringes and/or polystyrene tubes.

The method of estimation and assay kit of the present invention will now be described by way of example only with particular reference to the accompanying diagrams in which,

Figure 1 is a typical response curve (standard curve) which may be used to ascertain the LH concentration in a given sample, and

Figure 2 is a precision profile (i.e. a plot of precision as a function of LH concentration) of the assay illustrated in Figure 1.

1. Preparation of monoclonal antibody ES1

The monoclonal antibody was prepared as follows.

The cell line ES1, making antibody against luteinising hormone, was produced according to the general methods of G. Kohler and C. Milstein, Nature (1975) *256*, 495—497, and G. Galfre, S. C. Howe, C. Milstein, G. W. Butcher and J. C. Howard, Nature (1977) *266*, 550—552.

Twelve week old Balb/c female mice were injected intrapertoneally (i.p.) with 100 μg highly purified human luteinising hormone (LH) in Freund's complete adjuvant. Ten weeks later they received a further i.p. injection of 50 μg LH in saline. Eleven weeks after this, a mouse with a circulating anitbody titre of 1/180,000 was given a final injection of 100 μg LH in saline i.p., and after three days the spleen was removed. The spleen cells were suspended in culture medium (RPM1 1640 buffered with 20 mM Hepes, and supplemented with 2 mM L-glutamine, 1 mM sodium pyruvate, 50 units/ml penicillin, 50 μg/ml steptomycin, 10% foetal calf serum and 5×10^{-5}M 2-mercaptoethanol), at a concentration of 5×10^6 cells per mml, and cultured in 75 cm^2 T flasks at 37° in a humidified 5% CO_2-in-air atmosphere for seven days.

The spleen cells were fused with the 8-azaguanine-resistant, non-secreting mouse myeloma cell line NS1 abbreviated from P3-NS1/1-Ag4-1(Kohler, S. C. Howe and C. Milstein, European Journal of Immunology (1976) *6*, 292—295). The method used for the fusion was as described by V.T.Oi and L. A. Herzenberg (1980) in "Selected Method in Cellular Immunology" Edition B. B. Mishell and S. M. Sjiigi, pp 351—373. This method was modified after S. Fazekas de St Groth and D. Scheidegger, J Immunol Meth (1980) *35*, 1—21. The fusion ratio was two myeloma cells to one spleen cell. The number of fused cells p r well of a 96-well culture plate was 2.2×10^5 which were add d to a feeder layer of 1.7×10^5 mouse thymocytes per well.

Polyethylene glycol 4000 (BDH Chemicals) was used as the fusing agent. The cells were placed immediat ly in medium containing hypoxanthine, aminopterin and thymidien (HAT) (J. W. Littlefield, Science (1964) *145*, 709—710), but not mercaptoethanol, and not fed for seven days.

The hybrid cells were cloned by limiting dilutions into 96-well plates containing 5×10^5, mouse thymocytes per well Clones were selected according to how wel the cells grew.

As the clone cells multiplied, there were transformed from 96-well culture plate to a 24-well culture plate at which stage aminopterin was omitted from the medium; and then to 25 cm^2 T flasks, when hypoxanthine and thymidine were gradually reduced and then omitted.

The cells were harvested, resuspended in Dulbecco's Minimal Essential Medium, and 2×10^6 cells were injected i.p. in 0.2 ml to a number of Balb/c mice which had been pretreated with 0.5 ml Pristane (2, 6, 10, 14-tetramethyl-pentadecane) i.p. ten and three days previously (M. Potter Physiol Rev. (1972) *52*, 631—719). Ascites developed in about ten days. The fluid was withdrawn from the abdominal cavity over a period of several days. It was found that pools of the later tappings provided antibody of considerably higher titre (4—10 mg/ml) than did earlier tappings.

The above procedure yielded a number of hybrid cell lines producing monoclonal antibodi s to LH, four of which were efficient producers of MAbs. Of these, the cell line producing the monoclonal antibody having the highest avidity for LH was selected for further study and designated anti-human LH to ES1.

Subsequent studies of the ES1 MAb revealed it to be conformational MAb for LH, reacting with an epitope which includes structures on both the alpha and beta subunits of intact hLH. Thus although a standard preparation of the alpha subunit (preparation 72/20 from National Institute of Biological Standards and Control, London UK) gave 2.5% of the potency of intact hLH, assays carried out on eluates of the preparation from a Sephadex (Registered Trade Mark) G100 gel filtration column showed that all of this activity was attributable to contamination with intact LH present in the preparation. Similarly a standard preparation of the beta subunit (preparation 71/342 also from NIBSC) had 4.5% of the potency of intack hLH, but again all this activity was present in the eluting position of intact hLH when this preparation was passed through the same Sephadex gel filtration column as used above. These results indicate that the ES1 MAb reacts with intact hLH and not with the biologically inactive alpha and beta subunits.

The immunoglobulin fraction was prepared from the ascites fluid by precipitation with 18% sodium sulphate, and the IgG fraction thereof was isolated by means of a Sephadex G200 column. This material was dialysed against water and freeze dried.

2. Preparation of Iodine-125: MAb ES1 (Labelled receptor)

MAb ES1 was radioiodinated to a specific activity of 8 to 10 Ci per g (approximately one atom of iodine-125 per molecule of IgG by the chloramine T procedure of W. M. Hunter and F. C. Greenwood (Nature (Lond.) (1962) *194,* 495) using 100 g protein, 1 mCi of $Na^{125}I$ (IMS/30: Radiochemical Centre) and 32 g chloramine-T. The sodium metabisulphite of the original procedure was replaced by cysteine (75 g in 100 l).

3. Preparation of antiserum to human chorionic gonadotrophin (hCG) immobilised on sephacryl (unlabelled receptor)

A. The Sephacryl gel, pre-swollen or swollen according to the manufacturer's recommendations, was thoroughly washed with deionised water in a sintered glass funnel (porosity 2). 15 ml (settled volume) of the gel was resuspended in 50 ml 5 mM sodium meta-periodate in 0.1M sodium acetate buffer pH 5.0, and oxidised, with end over end mixing in a stoppered glass tube, for 1 hour at 15°C. Any remaining periodate was consumed by the addition of 5 ml 10% (v/v) glycerol with further mixing for 1 hour. Finally the gel was washed thoroughly with 2 litres 0.1M $NaHCO_3$ (pH 9.0).

B. Antiserum to the beta subunit of hCG (1 ml) was brought to 18% (w/v) Na_2SO_4 in 0.1M $NaHCO_4$ by the addition of anhydrous material with stirring at 25°C over 30 minutes. After standing for a further 1 hour the precipitate was collected by centrifugation (2000×g) for 15 minutes at 25°C and washed twice with 18% (w/v) Na_2SO_4 in 0.1M $NaHCO_3$ (5 ml). The washed precipitate was dissolved in 0.1M $MaHCO_3$ (1 ml) and then dialysed against 5 litres of 0.1M $NaHCO_3$ for 16 hours at 4°C before stored at −20°C until required.

C. The oxidised gel from step 3A above) was suspended in 20 ml 0.1 M $NaHCO_3$ (pH 9.0) containing IgG obtained, by the process of 3B, above, from the equivalent of 4 ml hCG antiserum (approximately 8—11 mg IgG per ml of gel). This suspension was mixed at 15°C for 16 hours before uncoupled protein solution was removed by filtering through a glass sinter (porosity 2). The coupled gel was resuspended in 50 mM phosphate buffered saline (PBS pH 7.5) and allowed to settle for 30 minutes before the supernatant was decanted to remove any fines. The coupled gel was resuspended in further PBS and added to 500 mg of solid sodium borohydride to give a final total volume of 100 ml. After allowing the reaction to proceed, with occasional stirring, for 30 minutes at 15°C, the reduced coupled gel (solid phase) was washed on a glass sinter, with PBS (2×100 ml) and with diluent containing 1% Tween-20 (Trade Mark) (5×100 ml). The solid phase preparation was then resuspended in 20 ml of diluent containing 1% Tween-20 and 0.01% sodium azide and stored at 4°C until required.

4. Immunoradiometric assay of LH standards

Human LH Standards (Preparation 68/40, available from the National Institute of Biological Standards and Control) in doubling doses over the −20°C range 0.625 to 640.0 kU/titre were stored at in assay diluent (tris phosphate 0.25M PH 8.5 containing 2% (w/v) horse serum and 0.1% (w/v) sodium azide). 100 l of this standard solution was dispersed into polystyrene tubes (75×12 mm) by means of a Hamilton Micro M dispenser. For the assay of serum samples the standards were made up in 67% bovine serum. ^{125}I-MAb (Example 2), 50 μl of a 400 ng/ml solution in assay diluent, was then added to the polystyrene tube by Hamilton repeating dispenser using a 21 g needle and the mixture was incubated for 2 hours at 15°C in an unstoppered tube on a horizontal orbital agitator (orbital diameter 2.5 cm at 260 rpm). Solid phase coupled antibody (Example 3, 50 μl was then added to the mixture using a Hamilton repeating dispenser using a 14 g needle, and the mixture was again incubated for 1 hour at 15°C. After this time free antibody was separated from bound antibody by the sucrose layering procedure described in UK 1,566,098 and US 4,125,375, using two consecutive sucrose washes, each of 2 ml and added over 15 seconds. Finally, the radioactivity of the bound antibody was counted for 1 minute. This procedure was repeated for a series of LH standards and the results are illustrated graphically in Figure 1.

Figure 1 provides a sample of the response curve which is made available by the present assay. The particular factors of the preferred embodiments of the present assay which determine the characteristics of the response curve, and hence the working range and detection limit of the assay are as follows:

i) the avidity of the MAb which is used for radioiodination;

ii) the process of radioiodination and method of storage of ^{125}I-MAb;

iii) the concentration of ^{125}I-MAb in the incubate;

iv) the avidity of the antibodies used to prepare the solid phase linked antibody;

v) the concentration of the solid phase linked antibody in the incubate, and

vi) the chemical procedure used to link the antibody to the solid phase support medium.

The response curve of Figure 1 is one of direct proportionality over the lower portion of its range, e.g. from 0.15—20 IU/ml and from about 0.15—17% bound. In the system described the non-specified response is consistently less than 0.1% of the count rate given by the ^{125}I-MAb present in the incubate.

The present response curve whose position (left to right in Figure 1) may be defined as having 10% maximum binding (itself about 80%) of total ^{125}I-MAb present in Figure 1) at about 12 IU/litre LH when present in a 100 μl serum sample in an incubation mixture of 200 μl, will give a detection limit which, in addition to the other factors specified above, is determined by the specific

activity of the labelled MAb, and the time which is allowed for measurement. A detection limit of 0.1 IU/litre or less is achieved with the present system when a 1 minute counting time is employed.

Figure 2 shows the precision profile of the assay illustrated in Figure 1 and indicates the relevant normal ranges for basal serum LH concentrations. The precision (CV) is that which applies to duplicate determinations, although it was calculated from quadruplicate measurements comprises of a duplicate standard curve at each beginning and end of an assay consisting of 180 tubes. Thus any within-assay drift which was present is represented in the precision estimates, and the assay is therefore very precise. In addition the variability of recovery of standard LH added at a concentration of 20 IU/litres serum to different samples of human serum is very small being typically in the region of 3—4%.

5. Immunoradiometric assay of patient's sample for LH

A serum sample (100 μl) from a patient with secondary amenorrhoea was dispensed into polystyrene tubes (75x12 mm) by means of a Hamilton Micro M dispenser. ^{125}I-MAb (Example 2), 50 μl of a 400 ng/ml solution in assay diluent, was then added to the polystyrene tube by Hamilton repeating dispenser using a 21 g needle, and the mixture was incubated for 2 hours at 15°C in an unstoppered tube on a horizontal orbital agitator (orbital diameter 2.5 cm at 260 rpm). Solid phase coupled antibody (Example 3, 50 μl) was then added to the mixture using a Hamilton repeating dispenser using a 14 g needle, and the mixture was again incubated on a horizontally orbiting agitator for 1 hour at 15°C. After this time free antibody was separated from bound antibody by the sucrose layering procedure described in UK 1,566,098 and US 4,125,375. Finally the radioactivity of the bound antibody was counted for 1 minute. By comparing the reading obtained with the response curve (Figure 1) the LH level in the patient's serum may be ascertained.

It is important to note that a response curve should be constructed for each assay, since the actual values will vary with the age of the reagents.

6. Alternative immunoradiometric assay of LH standards

The procedure of Example 4 was repeated except that instead of separately incubating the standard LH solution with ^{125}I-MAb and the solid phase coupled antibody in a two-stage process, the standard LH solution was incubated on a horizontally orbiting agitator (for 1 hour) with both ^{125}I-MAb and the solid phase coupled antibody in a single stage process. A response curve (not shown) with characteristics similar to those of Figure 1 (except that it was shifted slightly to the right) was obtained.

7. Alternative immunoradiometric assay of patient's sample for LH

The procedure of Example 5 was repeated except that instead of separately incubating the patient's serum sample with ^{125}I-MAb and the solid phase coupled antibody in a two-stage process, the patient's serum sample was incubated on a horizontally orbiting agitator (for 1 hour) with both ^{125}I-MAb and the solid phase coupled antibody in a single stage process. The LH level in the sample was ascertained from the response curve of Example 6.

The assay of the present invention has been found to be highly specific. Thus, for example, when the method of the invention was carried out on standard preparations of human follicle stimulating hormone (FSH) and human thyroid stimulating hormone (TSH), these were found to exhibit relative potencies of less than $^1/_{200}$ and less than $^1/_{50}$ respectively of that of LH. It is believed, however, that these results are due to contamination of the standard FSH and TSH preparations by LH. In further studies it has also been found that MAb ES1 does not bind to I-125/FSH or I-125/TSH.

The advantages of the present assay system, especially when MAb ES1 is employed include

a) a detection limit of 0.1 IU/litre serum or less in an assay which employs a 100 μl serum sample and only 1—3 hour incubation time;

b) a very wide working range of at least 0.1 to 200 IU/litre serum without recourse to the use of different serum sample volumes;

c) a single, general purpose assay is provided which can be used for all the present clinical purposes to which assays are put and which can provide the results within one working day, and

d) the need for the expensive and invasive LRF stimulation test is obviated by providing accurate and reliable basal LH measurements.

Claims

1. A monoclonal antibody produced by a hybrid cell line wherein the antibody has specificity for human luteinising hormone (LH).

2. A monoclonal antibody according to Claim 1, produced by a hybrid cell line derived from the fusion of a parent myeloma cell line with a spleen or other immunocyte cell imunised with LH.

3. A monoclonal antibody according to either Claim 1 or Claim 2, wherein the antibody reacts with an epitope and which includes structures on both the alpha and beta subunits of intact LH.

4. A monoclonal antibody according to any on of Claims 1 to 3, wherein the antibody is an IgG antibody.

5. A monoclonal antibody according to any one of Claims 1 to 4, produced by a hybrid cell line having the identifying characteristics of anti-LH No ES1, deposited with the Pasteur Institut under deposition No CNCMI-185.

6. A immunoassay method for the estimation of human luteinising hormone (LH) present in a sample comprising:

a) binding LH present in the sample to both a

labelled receptor and an unlabelled receptor to form a solid labelled complex, and

b) analysing for either labelled receptor bound in the solid labelled complex or residual unbound labelled receptor, wherein the labelled receptor is a monoclonal antibody according to Claim 1 bound to a label.

7. An assay kit for use in the estimation of human lutenising (LH) comprising:

a) a monoclonal antibody according to Claim 1 bound to a label, and

b) an unlabelled receptor which binds to LH to form a solid unlabelled complex.

8. An immunoassay method according to Claim 6 or an assay kit according to Claim 7, wherein the monoclonal antibody is an antibody according to Claim 2.

9. An immunoassay method according to Claim 6 or 8 or an assay kit according to either Claim 7 or 8, wherein the monoclonal antibody is an antibody according to Claim 3.

10. An immunoassay method according any one of Claims 6, 8 or 9 or an assay kit according to any one of Claims 7 to 9, wherein the monoclonal antibody is an antibody according to Claim 4.

11. An immunoassay method according to any one of Claims 6 or 8 to 10 or an assay kit according to any one of Claims 7 to 10, wherein the monoclonal antibody is an antibody according to Claim 5.

12. An immunoassay method according to any one of Claims 6, or 8 to 11 or an assay kit according to any one of Claims 7 to 11, wherein the monoclonal antibody is labelled with a radioactive label.

13. An immunoassay method according to any one of Claims 6, or 9 to 12 or an assay kit according to any one of Claims 7 to 12, wherein the unlabelled receptor is antiserum to human chorionic ganodotrophin (hCG) bound to a solid phase support.

14. An immunoassay method according to any one of Claims 6, or 8 to 12 or an assay kit according to any one of Claims 6 to 10, wherein the unlabelled receptor is antiserum to LH bound to a solid phase support.

15. An immunoassay method according to any one of Claims 6, or 8 to 12 or an assay kit according to any one of Claims 7 to 12, wherein the unlabelled receptor comprises a monoclonal antibody to hCG bound to a solid phase support.

16. An immunoassay method according to any one of Claims 6, 8 to 12 or an assay kit according to any one of Claims 7 to 12, wherein the unlabelled receptor comprises a monoclonal antibody to LH bound to a solid phase support, provided that said monoclonal antibody reacts with a different epitope than the antibody used as the labelled receptor.

17. An immunoassay method or assay kit according to any one of Claims 13—16, wherein the solid phase support is a copolymer of agarose and acrylamide.

18. An immunoassay method according to any one of claims 6 or 8 to 17, wherein the labelled receptor and unlabelled receptor are bound to the LH in an aqueous medium to form a solid complex and the solid complex is separated from the aqueous medium to facilitate analysis of the labelled receptor either in the bound complexed state or in the residual unbound form in solution in the aqueous medium.

19. An immunoassay method according to Claim 18, wherein the solid complex is separated from the aqueous medium by sucrose layering technique described in UK Patent No. 1566098.

20. An immunoassay method according to either Claim 18 or 19, in which amount of label present in the separated solid complex is determined.

21. An immunoassay method for the estimation of human luteinising hormone (LH) substantially as hereinbefore described with particular reference to either of the Examples 5 to 7 and to either of the Figures.

22. An assay kit according to any one of Claims 7—17, further comprising LH standard solutions.

23. An assay kit according to any one of Claim 7—17 or 22, further comprising a sucrose solution.

24. An assay kit for use in the estimation of human luteinising hormone substantially as hereinbefore described.

Printed for Her Majesty's Stationery Office by the Courier Press, Leamington Spa, 1983. Published by the Patent Office, 25 Southampton Buildings, London, WC2A 1AY, from which copies may be obtained